Experimental Biotechnology
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Lecture - 38
Polymerase Chain Reaction (Part – 1)

Hello everybody, this is Dr. Vishal Trivedi from department of bioscience and bioengineering IIT Guwahati and today we are going to start a new module and our new module is going to deal with the molecular biology tools. So, the molecular biology tools normally deals with the molecules which are important for the livelihood or for the maintenance of the life. So, there are 4 major molecules which are important for maintaining the life one its DNA, the second is RNA, the third is protein and the fourth is the lipids.

So, molecular biology normally deals with all the macromolecule especially the DNA, RNA and proteins. So, when we talk about a DNA is DNA is been amplified with a technique known as a polymerase chain reactions.

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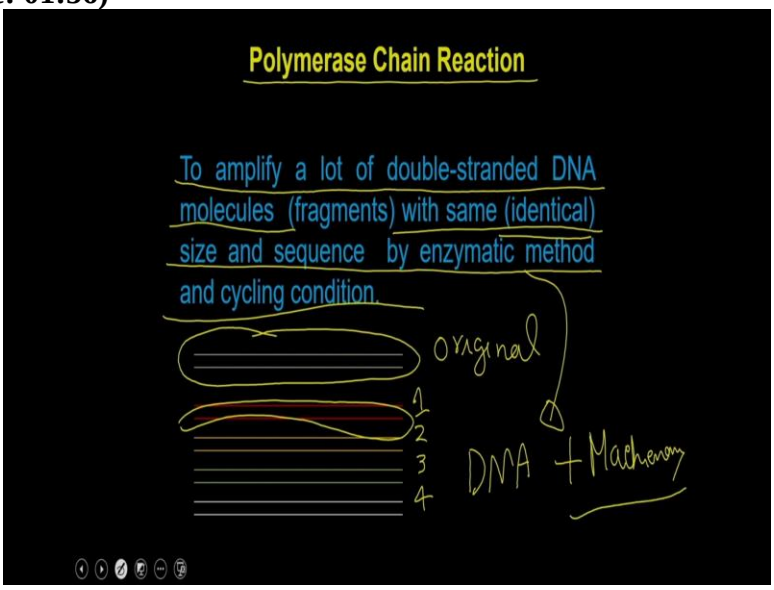

So, the, as the name suggests, the polymerase chain reaction is a technique which is used to amplify a lot of double-stranded DNA molecules with the same identical size and the sequence by the enzymatic method and the cyclic conditions. What it means is that you have original DNA, which is the original DNA and this original DNA is being amplified for multiple cycles.

And that is how you are actually going to have the multiple copies like you have the 4 multiple copies which are actually going to be identical.

Which means the sequence of the all these fragments are going to be identical to the original sequence and also they are going to be amplified with the help of the enzymes. So, if you want to understand the process of polymerase chain reactions, you have to understand the 2 basic components. One, you have to understand about the DNA. The second you have to understand about the enzymatic machinery, what is important for amplifying the DNA?

So, let us start with understanding the DNA first and then we are going to understand about the machinery and then subsequently we are also going to understand how people have developed the technique and then we are also going to understand all the technical aspects related to polymerase change reactions.

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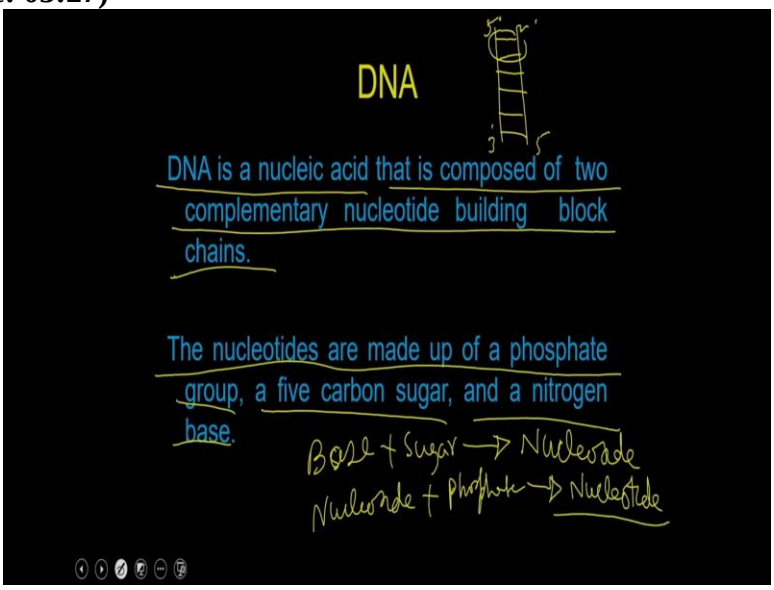

So, DNA is a nucleic acid and that is composed of the 2 complimentary nucleotide binding blocks chains which means in a typical DNA, you have the 2chains, one is the 5 prime to 3 prime and the 3 prime to 5 prime. Both are these strands are connected by the nucleotides which is present inside and the nucleotide are made up of a phosphate group, 5 carbon sugar and a nitrogen base.

So, as you know that when the base is adding up with the sugar, it is actually forming the nucleoside. And when the nucleoside is being attached with the phosphate, it is called as nucleotide. So the DNA is made up off of the nucleotides. So, it is actually a macro molecule where you have the 1 chain of the nucleotides connected to another chain of nucleotides and these 2 new chains of the nucleotides are complimentary to each other.

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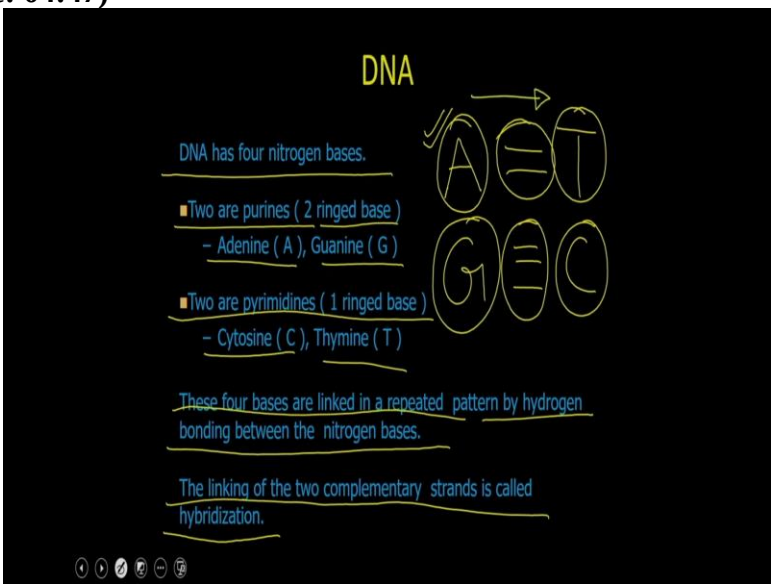

So, DNA has the 4 nitrogen bases, you have the 2 purines like the 2 ringed bases like adenine and guanine. And then you have the 2 pyrimidines like cytosine and thymine. These 2, 4 bases are linked in a repeated pattern by hydrogen bonding between the nitrogen bases. The linking of the 2 complimentary strands is called as the hybridization. So, A is making a pair with T and G is always making a pair with C.

So, what you see is that the A is making a pair with T with the help of the 2 hydrogen bonding whereas the G is making our hydrogen bonding with C with the help of the 3 hydrogen bonding which means, the strength of the A to T interaction is weaker compared to the interaction of G to C.

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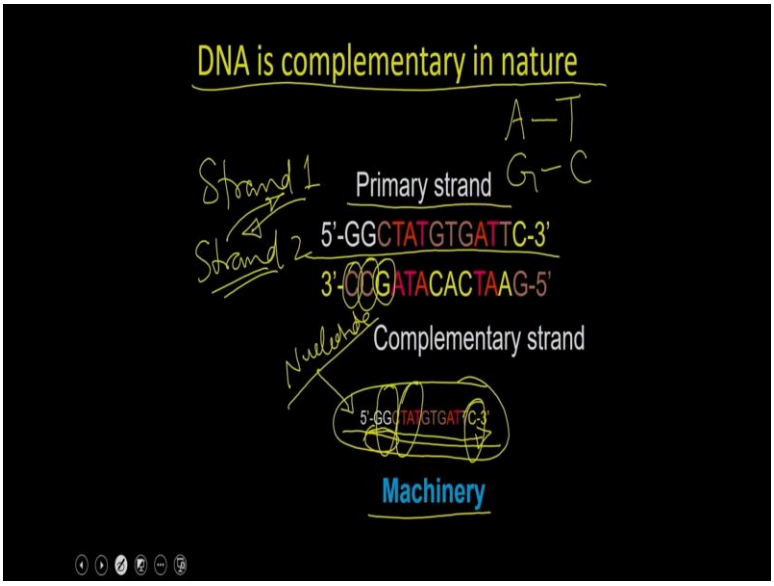

And because the A is making a pair with T and G is making a pair with C, the DNA is complimentary in nature. What is mean by complimentary in nature is that if you have a first sequence which is called as the primary strand then you can be able to deduce the complimentary sequence. For example, in this case, we have a primary sequence which is GGC TAT GTG and what you see is that wherever you have the G is actually having a C at a complimentary strands.

So, that is that is the way it is actually going to maintain the complementarity. How the complementarity is going to help you in terms of the you know come conserving the molecules because, if you have the strand 1, you can be able to generate the strand 2, because the strand 1 is complimentary to each other or if you have the strand 2, you can be able to generate a strand 1 and that is the basic principle to which the PCR mechanism is working.

For example, if I have this particular sequence and if I want to synthesize the strand 2 what I can do is I can simply attach a short nucleotide bases and then I can just put T machinery and that machinery is actually going to synthesize the complimentary sequences which means, I am looking for a machinery which actually can recognize this DNA sequence and then that machinery could be able to understand that the DNA is complimentary in nature.

So, what will happen is, if I have this sequence, the machinery is going to sit onto this sequence and then it is actually going to accept the nucleotide from what you are going to supply and then it is actually going to synthesize the complimentary strand. And once it reaches to the end of the

sequence, it will know that now, the synthesis is over. So, it is actually going to terminate. So, now, so far what we have discussed we have discussed about the molecule which is actually going to be amplified during the polymerase chain reactions.

And now, what we are going to understand we are going to understand about the machinery for the DNA synthesis machinery is the basis of the life on the earth or the DNA synthesis machinery is very crucial for duplicating the DNA.

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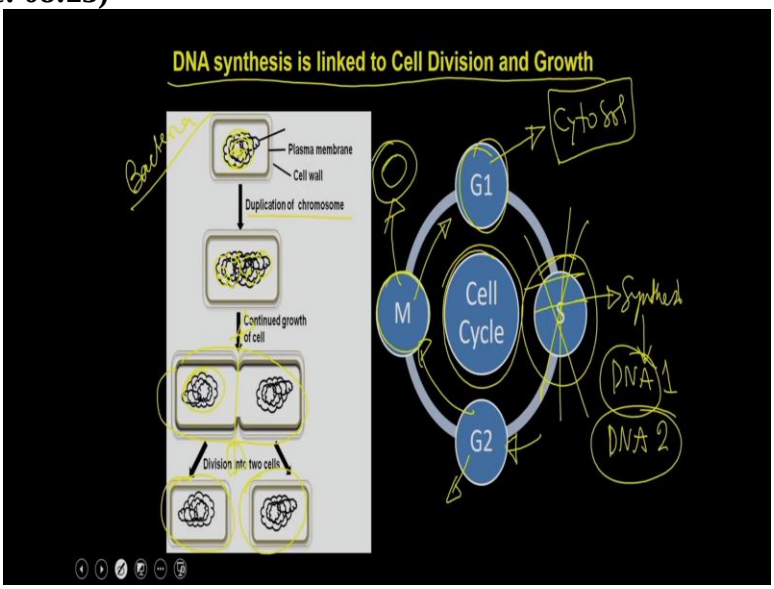

And DNA synthesis is linked to the cell division as well as the growth. So this is an example of the bacteria. So, what you see is that we have the bacteria which is actually having a single gene genome copy. And what will happen is that the first event itself is that the genome is actually going to duplicate into 2 copies. So, ultimately it is going to have 2 copies of the genome and then it is actually going to be divided by longitudinally and that is how you are actually going to have the 2 bacterial colonies.

Similarly, for in the prokaryote, it has the multiple events through which it actually goes through and then it is actually going to be divided into 2 and that all events are actually being called as the cell cycle. What are these events? You have a G1. So, in the G1, you are actually going to increase the amount of cytosol and you are actually going to prepare the cell for the synthesis phase and then you are actually entering into a synthesis phase.

So, in the synthesis phase or the S phase, you are actually going to do a DNA synthesis which means you are going to make a first copy of DNA and you are going to synthesize the second copy of DNA which means now the DNA is going to be duplicated which means now you are going to have the 2 genomes. Just like in this stage and now it will enter into the G2 phase. G2 is also preparative stage.

So, here also there will be some additional preparative stages. And then the cell will enter into the mitotic phase. And in this phase, the cell is going to divide into 2. So the original cell will again go through with the cell cycle whereas it is actually going to give you the 1 more copy of the cell. For that if you actually block the DNA synthesis, if you block this step, you are actually going to disrupt the cell cycle. And that is how you are actually going to stop the multiplication as well as the growth of particular cell.

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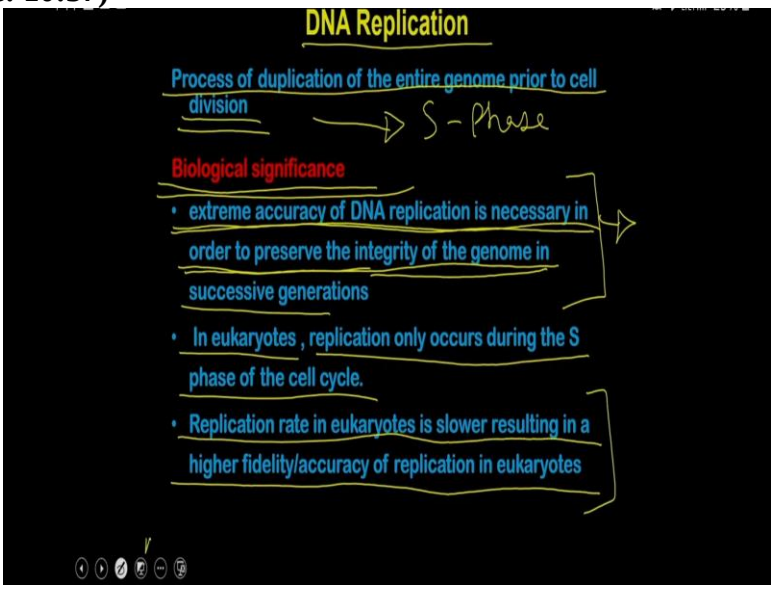

So, process of duplication of the entire genome prior to the cell division. So DNA replication is a process which occurs in the cell prior to the duplication of the cells. And that occurs within the S-phase. So it has a biological significance that the DNA replication is a very accurate process. And it requires to, in order to preserve the integrity of the genome in a successive generation which means if you are actually going to generate the mutations.

Or if you are start generating the modulations within the sequence of the genome, what you got from the original cell, you are eventually going to keep altering the DNA sequences and that

actually is not going to you know, carry the same information because the major function of the DNA is to carry the genetic information. So that it is actually going to allow the daughter cells to keep running the similar kind of metabolism.

So that is why it is important that DNA replication should be accurate. So that it should produce the identical copy in order to preserve the sequence of the DNA as well as it should preserve the integrity of the genome. And that should continue for several generations. In eukaryotes, the replication only occurred during the S phase of the cell cycle. The replication rate in a eukaryote is slower resulting in a higher fidelity accuracy of replication in eukaryotes.

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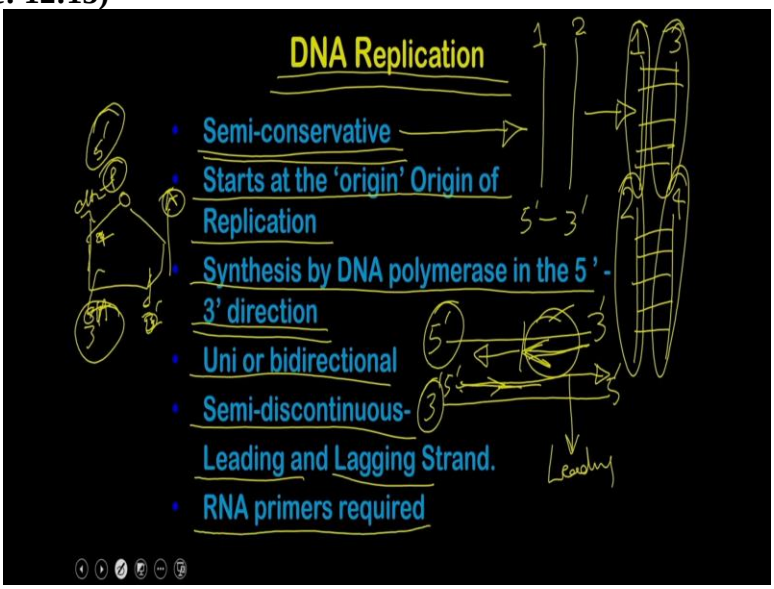

Now, the DNA replication is having the various characteristic. For example, DNA replication is semi conservative which means the DNA replication is going to be semi conservative means when you are actually going to synthesize for example, if this is the 2 strand 1 and 2, it is actually going to give you the 4 copies of 4 strands. But in this 4 strand, the 1 and 2 are going to be separated and the 3 and 4 are actually going to be synthesized.

So, the new strand, what is been synthesized is actually going to make a pair with the original strands and this process is called as the semi-conservative modes. So this is the 1 and 2 are actually coming from the parents whereas the 3 and 4 are actually the newly synthesized strains. This means the information what you have in the original DNA is going to be divided into 2.

And that is why it is not going to be conservative which means it is not going to make the pair like 1, 2 and 3, 4. It is actually going to make a pair like 1 and 3 and 2 and 4.

That is actually a semi conservative mode of replications. If you want to study more about or if you are interested to study more about how people have discovered the semi-conservative mode, you can easily go through some of the molecular biology book and you can read about the some of the classical experiment what has been designed and how people have discovered that the DNA replication is semi conservative in nature. It starts at the origin of the replication.

For DNA synthesis does not start at random places, it requires a place where it actually can start so that place is called as the origin of replications. In the bacterial cell, you have the single origin of replication whereas in the eukaryotic cell, you have the multiple places of the origin of replications because the bacterial genome is smaller in nature. So, it actually does not require the multiple origin of replications compared to that the eukaryotic genomes are bigger in size.

So they require the multiple origin of replication. So that the; application can start at multiple places. And that is how it actually can be able to complete the replications in a given timeframe. Because you know that the replication is important for making a second copy of the genome. So that the cell will enter into the G2 and M phase and that is how it actually going to divide and give you the more number of cells.

So that is why the and as phase is actually the bottleneck of the whole process and that so the DNA replication has to be in synchronous with the mitotic as well as the G2 phase. DNA synthesis by the DNA polymerase always occurs in the direction which is called as the 5 prime to 3 prime directions. So, you know that that DNA is actually a polymer of the nucleotides and nucleotides have 2 strands like 5 prime and 3 prime.

So, if I draw a typical nucleotide structures, what you will see is that this is actually a sugar then this is the base. So, for example, I have put A and here you have the sugar and on this fourth carbon, you have the fifth carbon and then you have the CH2 and then you have the phosphate group. So, this is actually the 5 prime end and this is the 3 prime end because here you have the

OH. No, this is the 3 prime end and this is the 2 prime end. So, this is actually you are going to have OH and that is OH, what you can actually be able to use.

So, if DNA synthesis is occurring, it is actually either utilizing this 5 prime OH or the 3 prime OH which means that is why, the DNA has a directionality which actually goes from 5 prime to 3 prime and 3 prime to 5 prime because this is the complimentary strand. So, the DNA synthesis always occurs in a direction which is called as the 5 prime to 3 prime which means it actually going to start the synthesis on the strand, the strand which is having the 3 prime ends.

And that is how it is actually going to synthesize in this direction because the sentences will be in the direction. It could be unidirectional or bi directional which means it can be either in this direction or it can be in both directions simultaneously. It is a semi discontinuous. For example, so, semi discontinuous means it is actually going to continue for some time and then it is actually going to stop and then it is again going to continue for that and that is why it is actually going to create the 2 different types of strands.

1 is called as the leading strand, the other 1 is called as the lagging strand. So, this is the strand which is called which is actually synthesizing the DNA in the multiple steps is called as the lagging strand whereas the strand which starts from the one end and goes continues like that is called as the leading strand. And for any DNA replication process, you require a primer and that primer is been synthesized by an enzyme which is called as the RNA primer. RNA primers and the primers which are been used in the DNA replication are made up off of the RNA and this has been synthesized by the RNA polymerase.

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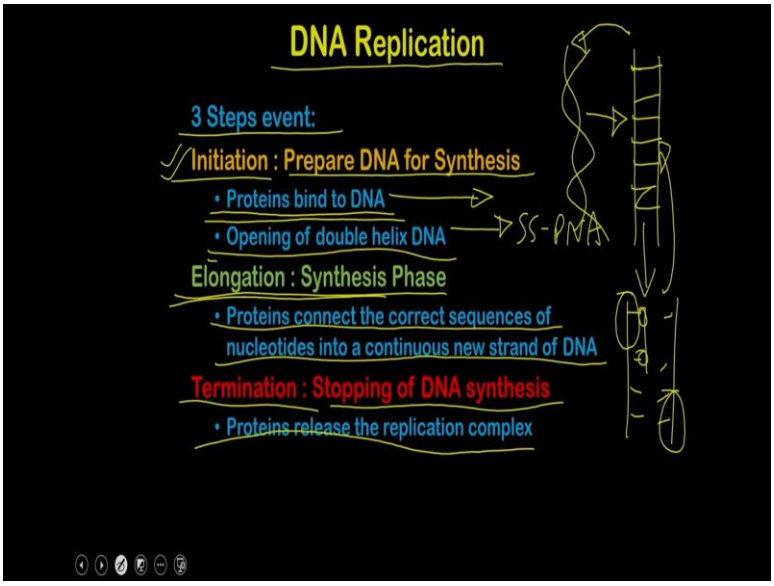

DNA replication has 3 steps. It has like initiation. So, in the initiation the DNA is going to be prepared for the synthesis. What is mean by the initiation is that in the initiation step the protein will bind to the DNA. So, you know, the DNA is a double helical structure. So, this double helical structure first has to be straight, so, that you first you have to remove the helicity of this particular DNA. So, you are going to first generate the DNA like double stranded DNA and then you have to actually break the 2 strands separately.

So, that it is actually going to be ready for accepting these this is machinery and that is what the events are happening when they are actually going for the initiation states. In the initiation states, the protein will bind to the DNA and then there will be an opening of double helix DNA. So, that it is actually going to be single stranded DNA. So, the single stranded DNA is going to be generated in the initiation stage then you have the elongation steps.

So, in the elongation steps, the DNA machinery is going to be set on to this and as well as onto this and then it is actually going to start adding the nucleotides and that is how it is actually going to start the synthesis of the second strands. So, in the elongation steps the protein will connect the correct sequence of the nucleotide into continuous new strands of DNA. And once the DNA machinery is going to be reached to the corners like the end of the sequence, it is actually going to stop the synthesis.

And that is what the third step that is third step is the termination which is actually going to stop the DNA synthesis. And once the DNA synthesis is over, the DNA has to again regain to its original form and that is the step what is required if you once you before you do the termination which means once the synthesis is over, the DNA is first going to be converted into a double helical structure and then from the double standard structure, you have to generate a double helical structure.

So, that is why DNA synthesis is a very, fine controlled process where you first have to do the initiation, where you the DNA has to be break into the 2 different strands and then the 2 different strands are going to be you know occupied by the DNA synthesis machinery and what the DNA synthesis machinery is going to do? So, DNA synthesis machinery involves the DNA polymerases which actually going to sit onto the enzyme.

And then they are actually going to read the original sequence and based on the original sequence, they are actually going to add the nucleotides on to the second strand and that is how it is actually going to start the synthesis of the second strand and once they reach to the end, that is what the elongation phase and once they reach to the end of the sequence, they are actually going to start the termination steps. So, once the synthesis is over, it is actually going to generate the double standard DNA and then double standard DNA is actually going to be coiled and then it is going to generate the helical structures.

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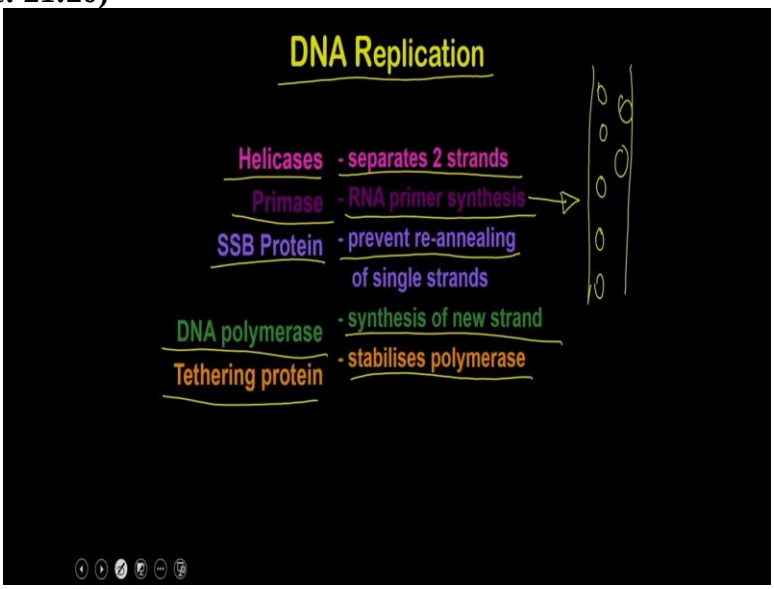

There are different enzymes what is involved in this DNA replication process. So, you require the helicases which actually going to separate the 2 different strands. Then you require the primase which is actually our RNA polymerase. So, it is actually going to be required for making a synthesis of the RNA primers. Then you require the single stranded DNA proteins. So, single stranded DNA proteins are actually going to bind to the 2 strands like this, so that they should not come together and again re-anneals.

And that is how it is actually going to maintain the single standard DNA. Then you require the DNA polymerase which is actually going to do a synthesis of new strand and then you required the tethering protein which actually going to stabilize the polymerases onto the strands.

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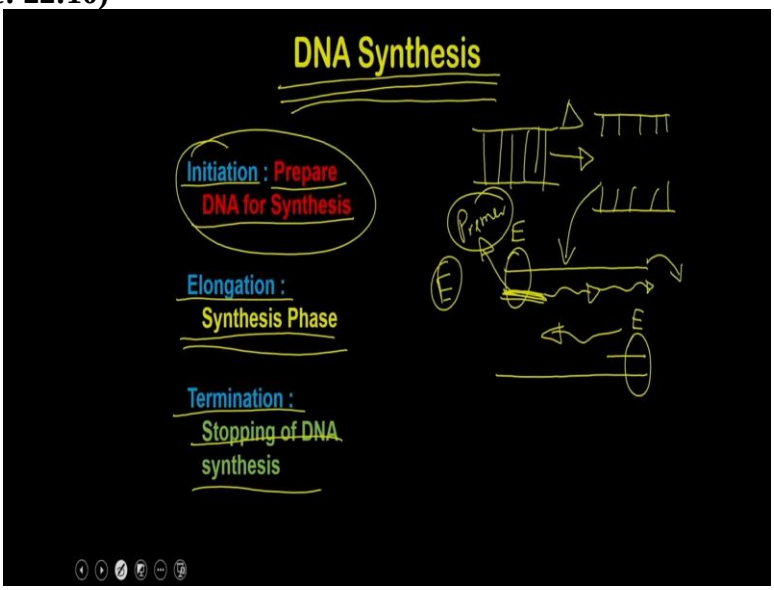

So, DNA synthesis has the 3 steps. 1 is called as the initiation which is actually going to prepare the DNA for the synthesis. So, what you are doing, you are actually taking a DNA and then you are actually generating the 2 strands then in the elongation steps, we are doing the DNA synthesis. So, once your 2 strands are ready, then what you can do is you are actually going to take and add the primers. So, in this case the RNA primers and so, the both the primers are going to be sit and then you are going to add the enzymes.

So the enzyme is going to be sit on to these strands. And that is how it is actually going to start the synthesis and once the termination, so you can stop the DNA synthesis. So, what will happen it will, once it will reach to this point, that enzyme is actually going to fall from the strands and

that it is actually going to complete the DNA synthesis and it will enter into the terminations. So now what you see is that these are the processes what we do under the DNA synthesis.

And what happens inside the cell can be mimic even outside the cell because all these events can be controlled even by without going through you know some of the enzymes what you require. For example, you require the helicases, so that it actually going to generate the single stranded DNA and that is how it is actually going to make the enough and then you require the single standard DNA binding proteins.

So that the DNA strands what have been generated by the helicases will remain as the single stranded. For these 2 processes can be simply be done by if you heat these particular strands. So if you heat this particular strand, what will happen is that the double standard DNA is going to be open into the single stranded templates. And that is how you can be able to utilize them. Now in the elongations phase, what you can do is you can simply add the small stretches of DNA sequences, which is actually going to be called as the primer.

And that is how it is and then you can add the enzyme. So, that is how you actually going to start the synthesis process. And once it reaches to the end, it is actually going to be a fall from the template and therefore it is actually going to end up into the terminations. So keeping these events and realizing that these events can be done under the invitro conditions. People have conceptualized the process of polymerase chain reaction and that is how they actually started developing the technique. But there are multiple steps and multiple phases in which the polymerase chain reactions is been developed.

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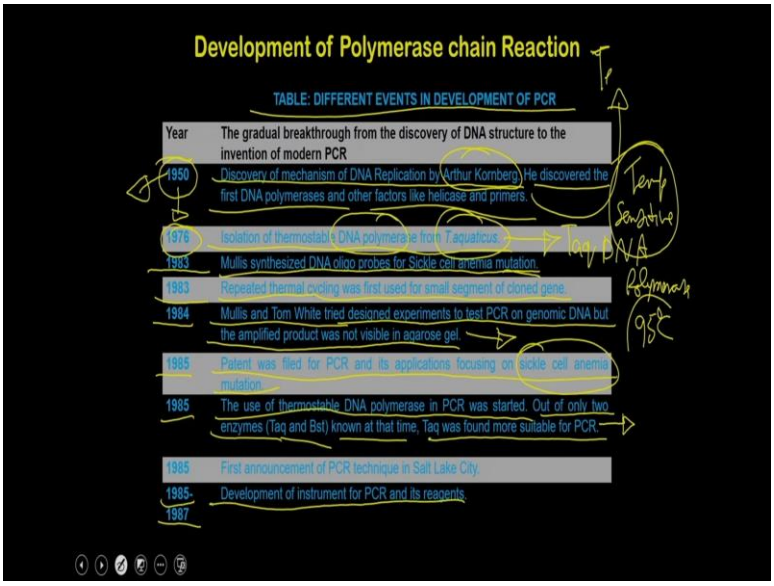

So, there are multiple events what people have done even to develop the PCR for the. So in 1950s people have discovered how the DNA replication is happening and that is being done by the Arthur Kornberg and he discovered the first DNA polymerase and other factors like helicase in and Primers. So, in 1950s when the Arthur Kornberg actually discovered the DNA polymerases as well as the mechanism of DNA replications and helicases and primases, that is how that the people know that the DNA is actually been replicated.

And it requires an enzyme and there is an enzyme. But what is the problem? The problem is the DNA polymerase what has been discovered by Arthur Kornberg is actually the temperature sensitive which means it is cannot withstand a very high temperature. And you know, that, as I said in the in the last slide itself, that when you want to mimic these conditions under the invitro conditions, the only way you can be able to escape the helicases and all other proteins is that you can actually heat up the DNA.

So that the DNA will come into the 2 strands and those 2 strands will remain as a single standard strands only if you keep the temperature very high. But once you bring the temperature high, the enzyme, what has been reported by Dr. Kornberg is actually going to be inactivated. And that is why nothing happened until in 1976 the people have discovered the first thermostable DNA polymerase from the thermo aquaticus and that thermo DNA polymerase is called as the taq DNA polymerase and that taq DNA polymerase is very, stable.

It can be withstand the temperature of 95 degrees Celsius which means you can be able to use this enzyme on multiple rounds. And you do not need to, you know, you do not need to add the enzyme on, you know, for every cycle you have to continue. Then in 1983, Kary mullis synthesizes that DNA oligo probes for sickle cell anemia. And then 1983, he only did the repeated thermal cycling was first used to clone a small segment of genome DNA.

And then in 1984, the Kary mullis and Tom white tried the design experiment to test PCR on the genomic DNA but the amplified product was not visible in the agarose. Then in 1985, the first patent was filed for its application regarding the detection of the sickle cell anemia mutations. And the 1985, the first use of thermostable DNA polymerase in PCR was started out of only 2 enzymes, the taq DNA polymerase, known at the time the taq was found more suitable for the PCR.

And 1985 to 1987 people have discovered a lot about the different types of instruments and so there are a lot of discoveries and development later on with the help of the instrumentations.

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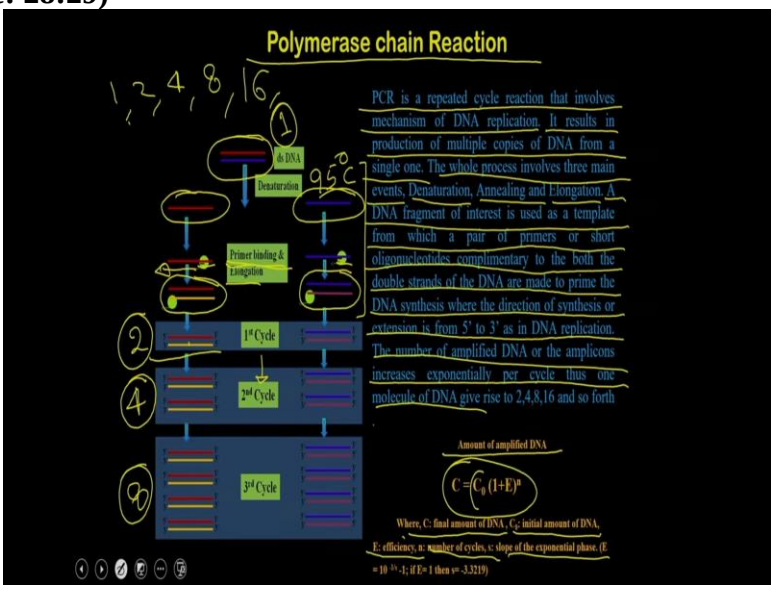

So what basically people are doing in a polymerase chain reaction. So PCR is a repeated cycling reaction that involves the mechanism of DNA replications. It results in the production of multiple copies of DNA from a single 1, the whole process involves 3 events, one is called denaturation, annealing, and the elongations. So what happens so as you can imagine that you started with original copy of the double stranded DNA.

So under the denaturation, when you have heated the sample at 95 degree Celsius, what will happen is that the 2 strands of the original copy are going to be separated and it will give you the 2 strands. Now what you are going to do is you are going to enter into the annealing phase where you are actually going to lower down the temperature. So, that the primers are going to buy and then you are actually going to have the primers.

So it is actually going to add the primers and that is how it is actually going to attach the primer on 1 end of this DNA as well as the 1 end of this DNA. And then the enzyme will enter into the elongation phase. And the enzyme is actually going to utilize this small primer DNA and that is how it is actually going to synthesize the whole strand and that is how you are actually going to get the double stranded DNA at the end.

So, this is actually is going to constitute the first cycle or the first cycle. After the first cycle, since you started with the 1 DNA, you are actually going to get 2 DNA. But as you can see, it is actually a semi-conservative, which means the 1 strand is from the original DNA. And the second strand is the newly synthesized DNA strand. Now, you can imagine that the same thing happened in the second cycle.

But now, this one also is these 2 strands are also going to serve as a template. And that is how you are actually going to get the 4 copies and in the third cycle, you are going to get the 8 copies which means a DNA fragment of interest is used as a template from which a pair of primer or short oligonucleotide complimentary to both the double strand of the DNA are made to prime the DNA synthesis where the direction of synthesis or the extension is 5 prime to 3 prime as it was in the case of DNA replications the number of amplified DNA.

Or the amplicons increases exponentially per cycle for example, if you start with the 1 DNA molecule, after first cycle, it is going to be to DNA, after second cycle it is going to be 4, after the third cycle it is going to be 8. So, if you continue that it is actually continuing like this cycle like so. So, it is actually doubling after every cycle and that is how it is actually going to give you very high amplification even from the 1 molecule.

So, if you imagine that if I started with 1 microgram of DNA, it is actually going to be several microgram of DNA and even within that 20, 25 cycles. So, the amount of amplified DNA you can be able to calculate simply by putting this formula that is the C is equal to C0 1 plus E to the power n where the C is the final amount of DNA, C0 is the initial amount of DNA, E is the efficiency and n is the number of cycles and S is the slope of the exponential phase and that you can be able to calculate from the this particular equations.

So, you can see that the polymerase chain reaction actually utilizes the similar concept what is happening in the during the DNA replications but instead of utilizing the multiple factors and such complicated mechanisms, what you are doing is you are simply heating the reactions, you are making the 2 strands, then you are adding the primers, which means the small DNA stretches and then you are asking to enzyme to utilize these primers to synthesize and then once the synthesis is over, then again you are bringing the temperature back and continuing the same cycle and that is how you are actually making the multiple reactions.

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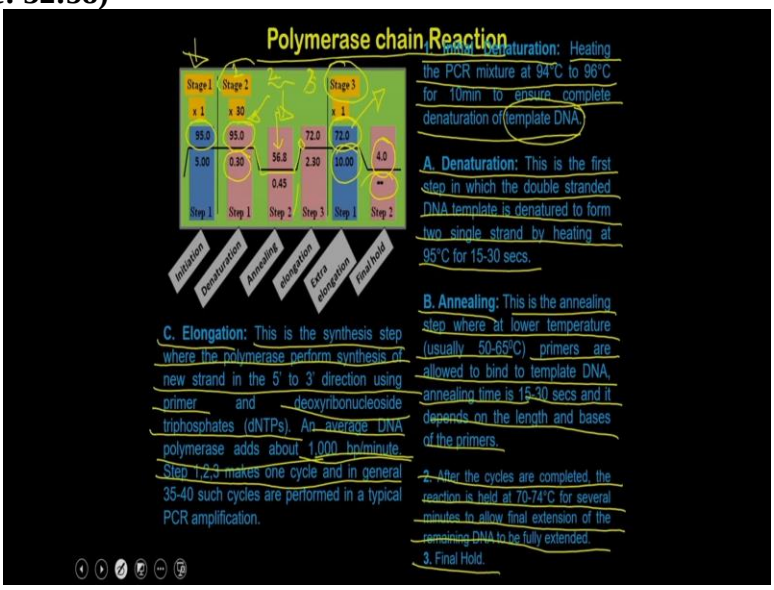

So the polymerase chain reaction requires the 4 events. So if you set up the polymerase chain reactions, you require the initial denaturation which means you have to heat the PCR mixture at 94 to 95th degree for 10 minutes to completely denature the template DNA. So when you are going to start the PCR reactions, you have to first bring the reactions and then you are going to

first do the initial denaturation where you are actually going to heat up the mixture at 95 degree Celsius for 5 to 10 minutes.

And then you are actually going to enter into the stage 2 where you are going to again heat up the template for 30 seconds to 45 seconds. And that actually is going to be our denaturation steps. So in the denaturation steps, this is the first step in which the double stranded DNA is going to be denature to form the 2 single standard DNA by heating the DNA at 95 degrees Celsius for 15 to 30 seconds. Now, you are going to lower down the temperature.

So that is going to be annealing steps. So in the annealing step, this is the annealing step where the lower temperature like 50 to 65 degree Celsius, the primers are allowed to bind to the template DNA and link time is 15 to 30 seconds and it depends on the length and the bases of the primers. So what you are going to do is you are you have lower down the temperature so that the primers which are actually been floating and which are small stretch of DNA are now go and bind to its complimentary DNA present onto the template.

And now, the enzyme will recognize this particular D complex and that is how the enzyme will start the synthesis. So after the annealing it will enter into the elongation phase. So this is the synthesis step where the polymerase chain performed the synthesis of the new strand in the 5 prime to 3 prime directions using the primers the dNTPs or the deoxyribo nucleotide triphosphates.

And average DNA polymerase adds about 1000 base pair per minute which means, after the annealing is over and the primer dimers has formed the complex, you are again going to increase the temperature. So that you will ensure that the template DNA should not bind to each other and then the enzyme will come sit onto these complexes and it is actually going to do a DNA synthesis.

And as an average 1000 base pair is going to be synthesized within the minute where if you supply the adequate amount of the deoxyribo nucleotides in the mixture. So, step 1 and 2, 3 will make 1 cycle. So this stage through means like step 1, 2 and 3 is actually constituting the first 1

cycle. And you can ask the machine to continue the cycle for another 30 rounds. And that is how it is actually going to make a lot of amplification.

Once that lot of amplification is over, then you can ask those things to enter into the next stage. And after the cycles are completed, the reaction is held at 70 to 74 degrees Celsius for several minutes to allow the final extension of the remaining DNA. And then you are actually going to ask to the final holds. So once stage 1, stage 2 is over, where you can actually do denaturation, annealing and elongations.

And you can do for multiple rounds then you can ask to enter into the stage 3, where you can just do another round of extension, which is called as the extended extensions for 10 minutes. So that whatever the replicons or whatever the amplicons are there in the reactions and which are not complete, by the end of this time, it is actually going to be completed in another 10 minutes. And then you can ask to for hold at 4 degree for infinite period of time.

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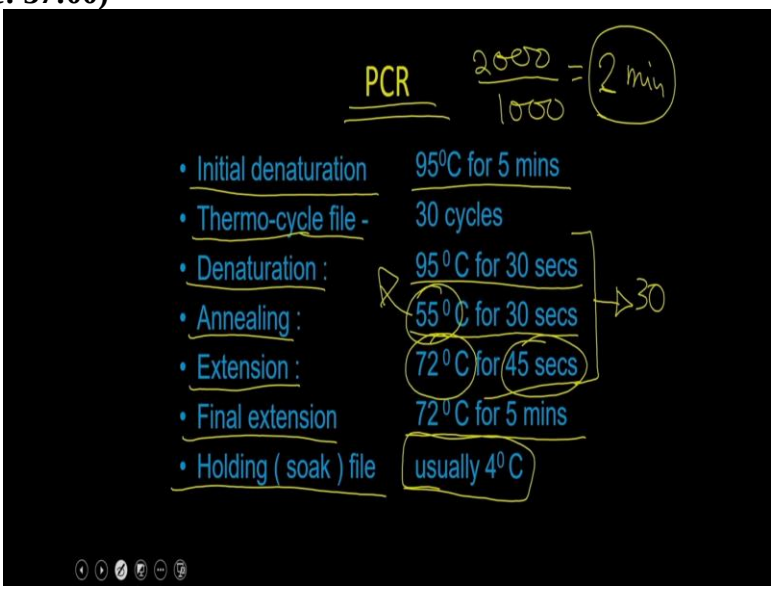

So what are the things you require for doing the PCR, So this is a classical example of 1 of the reaction cycles like you first you are going to do initial denaturation for at 95 degrees Celsius for 5 minutes. And then you are going to have a thermal cycling like these are the things are going to continue for the 30 cycles. And so in the 30 cycle, you are going to have the initial denaturation, like the 95 degree Celsius for 30 seconds, then you are going to have the annealing which is going to be 55 degrees Celsius for 30 seconds.

So this is not fixed, this has to be decided based on the TM as well as the annealing temperature of the primer dimers and all that kind of parameters. And then you are going to have the extension. So the extension will be at 72 degrees Celsius for another 45 seconds. So extension time is also been calculated based on the size of the target DNA because as you might have seen that the 1000 base pair is going to be synthesized in 1 minutes which means you can simply divide your target DNA by the 1000 and then you can put those many number of minutes.

For example, if I am amplifying a DNA which is of 2 KB, then what I can do is I can simply give the extension time slightly above to the 2 because the 2000 is being divided by 1000. And that actually is going to give you the 2 minutes, but it should be slightly bigger to the 2 minutes. So what I will do is I will give 2.1 or 2.2. So that there will be enough time for the enzyme to complete the extension process.

Otherwise, there will be lot of amplicons are going to be there which are not being complete. And then you are going to have the final extension. So final extension would be for 72 degrees Celsius for 5 minutes, and then there will be a final hold where you are actually going to ask the machine to keep the reactions at 4 degree because the DNA is very stable at 4 degree it cannot be remained stable at higher temperature. So you can ask them you know the samples to be stored at 4 degree.

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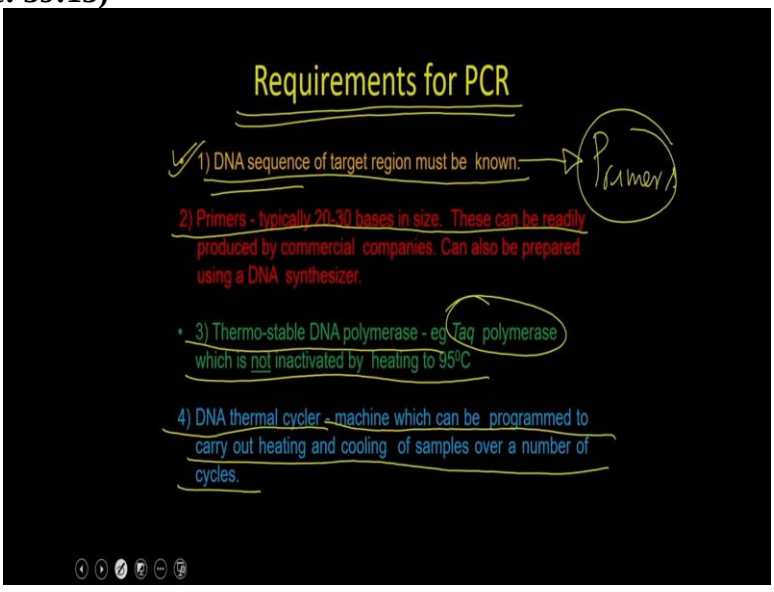

There are requirements for the PCR. So, before you start the PCR you should know all this then only you can be able to do the PCR. What is this requirements? The first requirement is that you should know the DNA sequence of the target DNA because if you do not know then you will not be able to synthesize the primers. Because this information is required to synthesize the primer and the primers are actually been the complimentary strands complimentary sequence to your target DNA.

Then you require the thermo stable DNA polymerase. For example, the taq DNA polymerase which is not inactivated by the heating at 95 degrees Celsius and then you require a thermal cycler which is machine which we have been used to programme to carry out the heating and cooling of the sample over a number of cycles. And with this, I would like to conclude my lecture here. Thank you.